NATURAL HETEROAGGLUTININS IN THE BODY-FLUIDS AND SEMINAL FLUIDS OF VARIOUS INVERTEBRATES[1]

ALBERT TYLER

William G. Kerckoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena

Introduction

Since the early work of Landois (1875) numerous investigators have noted in the normal serum of various species of animals, particularly among the vertebrates, the occurrence of agglutinins that act on the cells of certain other species (cf. Wiener, 1943; Landsteiner, 1945; Thomsen, 1932). Such natural heteroagglutinins have also been frequently reported to occur in the serum or body-fluids of various invertebrates (see literature in Huff, 1940; Tyler and Metz, 1945). Heteroagglutination reactions are frequently encountered in fertilizin studies and have been reported by several investigators (Lillie, 1913, 1919; Glaser, 1914; Just, 1919, 1930; Sampson, 1922; Godlewski, 1934; Hartmann *et al.*, 1940; Runnström *et al.*, 1944) as occurring between spermatozoa and foreign egg-water preparations, body-fluids and spermatozoa or their extracts. Further information concerning the range and nature of the heteroagglutination reactions is of importance, then, in analysis of problems of fertilization particularly in regard to the specificity and role of the interacting substances that are obtained from eggs and sperm.

A study of heteroagglutination reactions with lobster serum (Tyler and Metz, 1945; Tyler and Scheer, 1945), which normally acts on the sperm or the blood-cells of a wide variety of species throughout the animal kingdom, showed that at least ten distinct relatively class-specific agglutinins are present in the serum of this species. This was determined by means of absorption tests. In the controls for those tests the supernatant fluids of the sperm suspensions used for absorption were also examined for agglutinating activity and it was found, particularly when the sperm had not been previously washed, that the fluids from sperm of some species were active on cells of certain other species. Tests were then made with the body-fluids and these, too, were found to be active.

The present paper reports the results of this examination of the body-fluids and the sperm-supernatants of various species of invertebrates for the occurrence of heteroagglutinins. The species examined were for the most part those that have been used or are potentially useful in fertilizin studies. In addition a few absorption tests were made with starfish body-fluid to determine whether or not its agglutinating activity is attributable to the presence of several heteroagglutinins, each with broad specificity such as was found in lobster serum.

Materials and Methods

The body-fluids of twelve species of animals among the annelids, echinoderms, mollusks and tunicates were examined for possible agglutinating action on sperm

[1] This work has been aided by a grant from the Rockefeller Foundation. I am indebted to Miss Margaret L. Campbell for technical assistance.

suspensions of various species of invertebrates and, in a few cases, on erythrocyte suspensions of various vertebrates. The body-fluids were obtained by incision into the body cavity or by insertion of a hypodermic syringe into the body cavity. In the case of Ciona the fluid was obtained directly from the heart. The fluids were clarified by centrifugation and tested in the manner previously described (Tyler and Metz, 1945). The supernatant fluids of sperm suspensions of eleven of the same species were also tested. These were obtained by centrifugation of approximately 10 to 20 per cent suspensions of "dry" sperm in sea water. They may be termed diluted seminal fluids.

Experimental Part

In all of the species that were examined the body-fluids were found to possess agglutinating activity for the cells of certain other species. The results are presented in Table I. In some species (e.g., 1, 6, 18, 19, 21) the fluids exhibited agglutinating action on the cells of most of the species that were tested. However, the fluids of

Table I

Heteroagglutinating action of body-fluid (b) and seminal fluid (s) of various invertebrates

Spermatozoa (species 1 to 25) or erythrocytes (species 26–34) of:	Fluids of:																					
	1		4		6	12		13		14		16		18		19		21	22		23	
	b	s	b	s	b	b	s	b	s	b	s	b	s	b	s	b	s	b	b	s	b	s
POLYCHAETS																						
1. *Chaetopterus variopedatus*	0	0	0	0	+	0	0	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0
2. *Halosydna johnsoni*		0	0	0	+	0	0	+	+	+	+			+	+							
3. *Sabellaria californica*	+	+	0	0		0	0	0	0	0	0	0		+		+	+	0			0	0
ECHIUROIDS																						
4. *Urechis caupo*	+	+	0	0	+	0	0	0	0	+	+	+	+	+	+	+	+	+	+		+	
5. *Thalassema* sp.	+	+	0	0	+	0	0	0	0	+	+	+	+	+	+	+	+	+	+			
AMPHINEURANS																						
6. *Mopalia muscosa*	+	+	0	0	0	+	0	+	+	0	0	0	0	+	+		+	0	+		+	+
7. *Ischnochiton magdalensis*	+	+	0	0	0	+		+	+	0	0	0		+	+		+	0	+			+
GASTROPODS																						
8. *Acmea digitalis*	+	+	+		0	0				0	0			+	0			0			+	+
9. *Lottia gigantea*	+	+	+	0	0	0	0	+		0				+				0	+			
10. *Tegula galena*	+	+	+		+	0					0			+	+			+	+			
11. *Astraea undosa*	+	+			+	0		+	0	0				+	+			+	+			+
12. *Megathura crenulata*	+	+	+	0	+	0	0	+	+	0	0	0	0	+	+	+		+	+		+	+
PELECYPOD																						
13. *Mytilus californianus*	+	+	0	0	+	0	0	0	0	+	+	+	+	+	+	+		+	+		+	
ECHINOIDS																						
14. *Strongylocentrotus purpuratus*	0	0	0	0	+	0	0	+	+	0	0	0	0	0	0	0	0	+	0	0	0	0
15. *S. franciscanus*					+	0		+			0	0	0	0	0							
16. *Lytechinus pictus*	0	0	0	0	+	0	0	+	+	0	0	0	0	0	0	0		+	0		0	
17. *Dendraster excentricus*		0	0	0	+	0			+	0	0		0	0	0				0			

TABLE 1—*Continued*

Spermatozoa (species 1 to 25) or erythrocytes (species 26–34) of:	Fluids of:																							
	1		4		6	12		13		14		16		18		19		21		22		23	
	b	s	b	s	b	b	s	b	s	b	s	b	s	b	s	b	s	b	s	b	s	b	s
ASTEROIDS																								
18. *Patiria miniata*	+	+	0	0	0	+	+	0	0	0	0	0	0	0	0	0			+	0	0	0	0
19. *Pisaster ochraceus*	+	+	0	0	0	+	+	0	0	0	0	0	0	0	0	0	0	+		0	0		0
20. *Astropecten armatus*		+		0					0	0	0	0	0	0	0								
HOLOTHURIOID																								
21. *Stichopus californicus*				0			0		0		0		0		0	0	0	0	0					
ASCIDIANS																								
22. *Ciona intestinalis*	+	+	0	0	+	0	0	0	0	0	0	0	0	+	+	+	+	+		0	0	0	0
23. *Styela barnharti*	+	+	0	0	+	0		0	0	0	0	0	0	+	+	+	+	+	+	0	0	0	0
24. *Ascidia ceratodes*		+	0	0		0		0		0				+	+			+		0	0	0	0
FISH																								
25. *Leuresthes tenuis*	0		0			0		0		0	0		0	0	0			+	+		+		
26. *Girella nigricans*	0								0	0	0		0	0	0		0	+	+				
AMPHIBIA																								
27. *Rana catesbiana*	+	+																						
28. *Bufo halophilus*		+		0		0			0		0				0		0		0		+		+
REPTILE																								
29. *Sceloporus occidentalis*	+	0									0	0			0	0		+	+		0		
BIRD																								
30. Chicken		+		0			0		+		0			+	0		0		+		0		0
MAMMALS																								
31. Guinea pig								+	+	0				+									
32. Rabbit									+		0												
33. Sheep		+		0			0	+	+		0		0	+							0		0
34. Man									+		0			+									

none of the twelve species examined were found to possess agglutinating action on all of the species that were tested.

With one exception no heteroagglutination reactions occurred with body-fluids and cells of animals belonging to the same taxonomic class. The exception to this consists in the agglutination of Sabellaria sperm by Chaetopterus fluid. It is of interest in this connection that these two genera are placed (Pearse, 1936) in separate subclasses (cryptocephala and phanerocephala respectively) of the polychaets. Sabellaria also differs from the other two polychaets that were tested in that its cells fail to agglutinate in the fluid of the mussel (13), sea-urchins (14, 16) and sea cucumber (21) as well as that of the lobster previously (Tyler and Metz, 1945) reported.

Another feature of the results is that closely related species behave alike with respect to the ability or inability of their cells to be agglutinated by the various body-fluids. This was evident in the previously reported experiments with lobster serum.

In the present data differences are observed between species belonging to different orders, as in the case of the two species (8 and 9) belonging to the docoglossid gastropods which differ from the three rhipidoglossid species (10, 11, and 12) in their reactions to two of the fluids (6 and 21). With the other twenty-nine species tested, similarity in behavior is exhibited by members of the same class or sub-class.

The tests with the diluted seminal fluids gave results that in most cases paralleled those obtained with the body-fluids. Eight exceptions (1 on 29; 4 on 9 and 12; 12 on 6; 13 on 11; 18 on 8 and 30; 21 on 14) may be noted in Table I out of a total of 186 cross-combinations in which both seminal fluid and body-fluid were examined. These exceptions are all in the same direction; namely, a failure of the dilute seminal fluid to cause agglutination while the corresponding body-fluid is active. It can, then, be stated that in those cases in which the diluted seminal fluid is found to possess heteroagglutinating activity, the corresponding body fluid is likewise found to be active. Thus, similarly acting heteroagglutinins are found in both body-fluid and seminal fluid. The above-mentioned few exceptions may be attributed to failure to obtain, in certain seminal fluid preparations, sufficient concentration of a particular heteroagglutinin to produce a visible reaction with the cells of some species. However, the tests necessary to determine the validity of this explanation have not, as yet, been made.

From the similarity in action of seminal fluid and body-fluid, it might be inferred that the activity of the former is due to contamination with the latter. However, it may be noted that in most of the species employed for preparation of seminal fluid (e.g., 4, 12, 14, 16, 18, 19, 21, 22) the sperm is readily obtained without any appreciable admixture of body-fluid. Another interpretation is that seminal fluid is normally similar to body-fluid in composition. Against this may be cited the fact that readily recognizable constituents of body-fluid, such as hemocyanin in the mollusks, are not observed in the seminal fluids. A third possibility is that identical heteroagglutinins are present in both fluids. However, serological similarity does not imply entirely identical molecular constitution. Reaction with a specific antigen implies similarity only on the part of the specific combining groups of the antibodies from diverse sources. In the present case it has not been shown that the heteroagglutinin in seminal fluid and that in body-fluid both react with the same antigenic group or structure on the sperm that they agglutinate. However, the generally parallel behavior of the two fluids, when tested with spermatozoa of different species, favors that view.

Absorption tests, to determine whether or not more than one heteroagglutinin is involved in the action of a particular fluid, were carried out with Patiria body-fluid. These were done in the manner previously described (Tyler and Metz, 1945). Before being used for absorption the sperm were washed repeatedly in order to free them of agglutinins contributed by the seminal fluid, and this was checked in each test by examination of the supernatant of an aliquot part of the sperm for agglutinating activity.

Samples of Patiria body-fluid were absorbed with sperm of six species of animals and tested for agglutinating activity on sperm of nine species. The results are given in Table II. This limited set of tests reveals the presence of at least four distinct heteroagglutinins in Patiria body-fluid. These evidently comprise:—one for the two polychaets, one for the two echiuroids and Mytilus, one for the two gastropods, and one for the two ascidians. It seems likely, then, that the situation in Patiria body-

fluid is similar to that previously reported for the lobster; namely, the presence of a number of distinct agglutinins, each with broad group specificity.

Discussion

Heteroagglutinins are evidently normal constituents of the body-fluids of animals. They have generally been considered to be non-specific agents. However, from the fact that the fluids of various species act on the cells of different assemblages of other species, the heteroagglutinins must be regarded as having some degree of specificity. The previously reported (Tyler and Metz, 1945) absorption tests with lobster-serum gave evidence of the presence of ten distinct heteroagglutinins which are, for the most part, each specific for a taxonomic class of animals. The present results with Patiria body-fluids are indicative of similarly broad specificity on the part of the four heteroagglutinins found therein. It is clear, however, that heteroagglutinins of different species and also different heteroagglutinins of the same animal may differ considerably in the range of species on which they act. The rule is

Table II

Agglutinative activity of Patiria body-fluid after absorption with spermatozoa of various species

Spermatozoa of:	Body-fluid absorbed with washed sperm of:					
	Chaetopterus	Urechis	Thalassema	Astraea	Megathura	Mytilus
Chaetopterus ..	0	+	+	+	+	+
Halosydna.....	0	+	+	+	+	+
Urechis.....	+	0	0	+	+	0
Thalassema....	+	0	0	+	+	0
Astraea.....	+	+	+	0	0	+
Megathura.....	+	+	+	0	0	+
Mytilus.....	+	0	0	+	+	0
Ciona.....	+	0	0	+	+	+
Ascidia.....	+	0	0	+	+	+

that closely related species react alike to a particular fluid, but the closeness of relationship required depends upon the particular heteroagglutinating fluid employed. In the present tests (Table I) we find that species that belong to the same class, in most instances, behave alike with respect to the ability or inability of their cells to be agglutinated by the body-fluids of all twelve of the species examined.

In the sera of various mammals natural heteroagglutinins are found (cf. Thomsen, 1932; Wiener, 1943; Landsteiner, 1945) that are relatively species-specific and in human sera, as is well known, natural isoagglutinins are encountered that differentiate groups of individuals. In the various invertebrate body-fluids examined, agglutinins of such specificity have not, as yet, been found, the fluid of a particular species being inactive on cells of closely related species. However, from the ripe gametes, of many of these species natural agglutinins are obtained that act within the species. These consist in the fertilizins from eggs and antifertilizins from sperm described by Lillie (1913 et seq.), Just (1930), Frank (1939), Tyler (1939 et seq.), Hartman *et al.* (1939 et seq.), Runnström *et al.* (1942 et seq.) and others. While these agents act on gametes of the opposite sex within the species, they also have

been found to act on closely related species and in some instances the preparations act on remotely related species. Thus Arbacia egg water was found (Lillie, 1913) to agglutinate Nereis sperm, and sea-urchin eggs have been found (Runnström *et al.*, 1942) to be agglutinated by sperm extracts of animals as remotely related as the salmon and the ox. In Lillie's experiment it was shown that absorption with Nereis sperm removed the cross-reacting substance from Arbacia egg water without diminishing its agglutinating action on Arbacia sperm. Similar absorption experiments have not been reported in most of the cross-heteroagglutination reactions obtained by later workers with fertilizin and antifertilizin preparations. The need for such experiments is quite evident before any adequate determinations can be made of the specificity of these interacting substances obtained from the gametes. The present results may provide a helpful basis of procedure in such experiments.

The bearing of the heteroagglutination reactions on phylogenetic questions has been previously (Tyler and Metz, 1945) discussed. The present results are consistent with the previously expressed view that the reactivity of the cells of a particular species with various fluids is a characteristic of a group of related species and constitutes a group-specific trait in addition to the various group-specific morphological and chemical features of animals. There is no reason, as yet, for considering this trait to be of more general significance than any other in any applications that might be made to phylogenetic problems.

Summary

1. The body-fluids of 12 species of invertebrates (including two ascidians) and the seminal fluids of 11 species were examined for agglutinating action on the spermatozoa or blood cells of 34 species of animals.

2. All of the fluids were found to contain agglutinins for the cells of some of the species tested. Five of the fluids gave reactions with most of the species but none reacted with all of the species.

3. With one exception no heteroagglutination reactions were obtained with fluids and cells of animals belonging to the same taxonomic class.

4. Closely related (same class in most cases or same order in some) species were found to behave alike with respect to the ability or inability of their cells to react to the various fluids, and the fluids of closely related species exhibited similar reactivity.

5. The diluted seminal fluids gave reactions that in most cases paralleled those obtained with the body-fluids.

6. Absorption tests revealed the presence of at least four distinct heteroagglutinins in Patiria body-fluid, and indicated that each is characterized by a broad group-specificity similar to that previously reported for lobster-serum.

7. The general bearing of these results on fertilizin-antifertilizin reactions and on phylogenetic problems is briefly discussed.

LITERATURE CITED

Frank, J. A., 1939. Some properties of sperm extracts and their relationship to the fertilization reaction in Arbacia punctulata. *Biol. Bull.*, **76**: 190–216.

Glaser, Otto, 1914. A qualitative analysis of the egg secretions and extracts of Arbacia and Asterias. *Biol. Bull.*, **26**: 367–386.

Godlewski, E., 1934. Nouvelles recherches sur l'héteroagglutination des spermatozoïdes et sur l'action d'extraits de cellules sexuelles d'espèces étrangères. *Arch. de Biologie*, **45**: 735–807.

HARTMANN, M., AND SCHARTAU, 1939. Untersuchungen uber die Befruchtungsstoffe der Seeigel. I. *Biol. Zentralbl.*, **59**: 571–587.

HARTMANN, M., O. SCHARTAU, AND K. WALLENFELS, 1940. Untersuchungen uber die Befruchtungsstoffe der Seeigel. II. *Biol. Zentralblatt*, **60**: 398–423.

HUFF, C. G., 1940. Immunity in invertebrates. *Physiol. Rev.*, **20**: 68–88.

JUST, E. E., 1919. The fertilization reaction in Echinarachnius parma II, the role of fertilizin in straight and cross-fertilization. *Biol. Bull.*, **36**: 11–38.

JUST, E. E., 1930. The present status of the fertilizin theory of fertilization. *Protoplasma*, **10**: 300–342.

LANDOIS, L., 1875. *Die Transfusion des Blutes.* Leipzig.

LANDSTEINER, K., 1945. *The specificity of serological reactions.* Harvard Univ. Press, Cambridge, Mass.

LILLIE, F. R., 1913. Studies of fertilization. V. The behavior of the spermatozoa of Nereis and Arbacia with special reference to egg-extractives. *Jour. Exp. Zool.*, **14**: 515–574.

LILLIE, F. R., 1919. *Problems of fertilization.* Univ. Chicago Press, Chicago.

PEARSE, A. S., 1936. *Zoological names. A list of phyla, classes and orders.* Prepared for section F, A. A. A. S. Duke University Press, Durham, N. C.

RUNNSTRÖM, J., S. LINDVALL, AND A. TISELIUS, 1944. Gamones from the sperm of sea urchin and salmon. *Nature*, **153**: 285.

RUNNSTRÖM, J., A. TISELIUS, AND S. LINDVALL, 1945. The action of androgamone III on the sea-urchin egg. *Ark. f. Zool.* (Stockholm), **36A**, No. 22: 1–25.

RUNNSTRÖM, J., A. TISELIUS, AND E. VASSEUR, 1942. Zur Kenntnis der Gamonwirkungen bei Psammechinus miliaris und Echinocardium cordatum. *Ark. f. Kemi* (Stockholm) **15A**, No. 16: 1–18.

SAMPSON, M. M., 1922. Iso-agglutination and hetero-agglutination of spermatozoa. *Biol. Bull.*, **43**: 267–284.

THOMSEN, O., 1932. *Serologie der Blutgruppen. Chap. 2 of P. Steffan's Handbuch der Blutgruppenkunde.* J. F. Lehmanns Verlag, Munich.

TYLER, A., 1939. Extraction of an egg membrane-lysin from sperm of the giant keyhole limpet (Megathura crenulata). *Proc. Nat. Acad. Sci.*, **25**: 317–323.

TYLER, A., 1942. Specific interacting substances of eggs and sperm. *West. J. Surg. Obst. and Gyn.*, **50**: 126–138.

TYLER, A., 1942. A complement-release reaction; the neutralization of the anticomplementary action of sea-urchin fertilizin by antifertilizin. *Proc. Nat. Acad. Sci.*, **28**: 391–395.

TYLER, A., AND C. B. METZ, 1945. Natural heteroagglutinins in the serum of the spiny lobster, Panulirus interruptus. I. Taxonomic range of activity, electrophoretic and immunizing properties. *Jour. Exp. Zool.*, **100**: 387–406.

TYLER, A., AND B. T. SCHEER, 1945. Natural heteroagglutinins in the serum of the spiny lobster Panulirus interruptus. II. Chemical and antigenic relation to blood proteins. *Biol. Bull.*, **89**: 193–200.

WIENER, A., 1943. *Blood groups and blood transfusion.* 3rd ed. C. C. Thomas, Springfield.